U.S. DEPARTMENT OF COMMERCE
National Technical Information Service
PB-290 322

Effect of Insecticides on Benzo(A)Pyrene Carcinogenesis

Jefferson Medical Coll, Philadelphia, PA

Prepared for

Health Effects Research Lab, Research Triangle Park, NC

Nov 78

United States
Environmental Protection
Agency

Health Effects Research
Laboratory
Cincinnati OH 45268

EPA-600/1-78-066
November 1978

Research and Development

PB 290322

EPA

Effect of Insecticides on Benzo(*a*)pyrene Carcinogenesis

REPRODUCED BY
NATIONAL TECHNICAL
INFORMATION SERVICE
U. S. DEPARTMENT OF COMMERCE
SPRINGFIELD, VA. 22161

RESEARCH REPORTING SERIES

Research reports of the Office of Research and Development, U.S. Environmental Protection Agency, have been grouped into nine series. These nine broad categories were established to facilitate further development and application of environmental technology. Elimination of traditional grouping was consciously planned to foster technology transfer and a maximum interface in related fields. The nine series are:

1. Environmental Health Effects Research
2. Environmental Protection Technology
3. Ecological Research
4. Environmental Monitoring
5. Socioeconomic Environmental Studies
6. Scientific and Technical Assessment Reports (STAR)
7. Interagency Energy-Environment Research and Development
8. "Special" Reports
9. Miscellaneous Reports

This report has been assigned to the ENVIRONMENTAL HEALTH EFFECTS RESEARCH series. This series describes projects and studies relating to the tolerances of man for unhealthful substances or conditions. This work is generally assessed from a medical viewpoint, including physiological or psychological studies. In addition to toxicology and other medical specialities, study areas include biomedical instrumentation and health research techniques utilizing animals — but always with intended application to human health measures.

This document is available to the public through the National Technical Information Service, Springfield, Virginia 22161.

TECHNICAL REPORT DATA
(Please read Instructions on the reverse before completing)

1. REPORT NO. EPA-600/1-78-066	2.
3. RECIPIENT'S ACCESSION NO. PB290322	
4. TITLE AND SUBTITLE: EFFECT OF INSECTICIDES ON BENZO(A)PYRENE CARCINOGENESIS	5. REPORT DATE: November 1978
	6. PERFORMING ORGANIZATION CODE
7. AUTHOR(S): Anthony J. Triolo	8. PERFORMING ORGANIZATION REPORT NO.
9. PERFORMING ORGANIZATION NAME AND ADDRESS: Jefferson Medical College, Thomas Jefferson University, Philadelphia, PA 19107	10. PROGRAM ELEMENT NO. 1EA615
	11. CONTRACT/GRANT NO. R-803486
12. SPONSORING AGENCY NAME AND ADDRESS: Health Effects Research Laboratory, Office of Research and Development, U.S. Environmental Protection Agency, Research Triangle Park, N.C. 27711 RTP,NC	13. TYPE OF REPORT AND PERIOD COVERED
	14. SPONSORING AGENCY CODE EPA 600/11

15. SUPPLEMENTARY NOTES

16. ABSTRACT

The pesticides parathion, toxaphene, and carbaryl were tested for their ability to induce tumors in the forestomach and lungs of female Ha/ICR and A/J mice respectively. None of these pesticides, when fed alone in the diet of the mice, showed significant oncogenic activity. On the other hand, toxaphene enhanced benzo(a)pyrene (BP)-induced tumors and increased BP hydroxylase activity in the forestomach of the Ha/ICR mice and carbaryl enhanced BP-induced tumors and increased BP hydroxylase activity in the lungs of the A/J mice. In each instance, it is possible that toxaphene and carbaryl exhibited a cooncogenic effect in enhancing the BP-induced tumors. Conversely, toxaphene decreased the incidence of BP-induced tumors and inhibited BP hydroxylase activity in the lungs of the A/J mice. These results suggest that increased BP hydroxylase activity in tissues tends to enhance tumor formation and a decrease in the enzyme activity may have a protective effect against tumors. The relationship between enzyme inducibility and tumor formation may be due to the level of oncogenic epoxides formed in target organs. Further, studies of the formation of specific oncogenic epoxides of BP in tissues after treatment with these pesticides would help towards defining more clearly the relationship between BP hydroxylase inducibility and BP oncogenesis.

17. KEY WORDS AND DOCUMENT ANALYSIS

a. DESCRIPTORS	b. IDENTIFIERS/OPEN ENDED TERMS	c. COSATI Field/Group
neoplasms pesticides carcinoid tumors toxicology	oncogenesis carcinogenesis benzo(a)pyrene hydroxylase aryl hydrocarbon hydroxylase	06 F, T

18. DISTRIBUTION STATEMENT: RELEASE TO PUBLIC	19. SECURITY CLASS (This Report): UNCLASSIFIED	21. NO. OF PAGES: 41
	20. SECURITY CLASS (This page): UNCLASSIFIED	22. PRICE: A03-A01

EPA Form 2220-1 (Rev. 4-77) PREVIOUS EDITION IS OBSOLETE

EPA-600/1-78-066
November 1978

EFFECT OF INSECTICIDES ON BENZO(A)PYRENE CARCINOGENESIS

by

Anthony J. Triolo
Jefferson Medical College of
Thomas Jefferson University
Philadelphia, PA 19107

Gr nt No. R-803486

Project Officer

Thomas M. Scotti
Toxic Effects Branch
Environmental Toxicology Division
Health Effects Research Laboratory
Research Triangle Park, N.C. 27711

U.S. ENVIRONMENTAL PROTECTION AGENCY
OFFICE OF RESEARCH AND DEVELOPMENT
HEALTH EFFECTS RESEARCH LABORATORY
RESEARCH TRIANGLE PARK, N.C. 27711

1a

DISCLAIMER

This report has been reviewed by the Health Effects Research Laboratory, U.S. Environmental Protection Agency, and approved for publication. Approval does not signify that the contents necessarily reflect the views and policies of the U.S. Environmental Protection Agency, nor does mention of trade names or commercial products constitute endorsement or recommendation for use.

FOREWORD

The many benefits of our modern, developing, industrial society are accompanied by certain hazards. Careful assessment of the relative risk of existing and new man-made environmental hazards is necessary for the establishment of sound regulatory policy. These regulations serve to enhance the quality of our environment in order to promote the public health and welfare and the productive capacity of our Nation's population.

The Health Effects Research Laboratory, Research Triangle Park, conducts a coordinated environmental health research program in toxicology, epidemiology, and clinical studies using human volunteer subjects. These studies address problems in air pollution, non-ionizing radiation, environmental carcinogenesis and the toxicology of pesticides as well as other chemical pollutants. The Laboratory participates in the development and revision of air quality criteria documents on pollutants for which national ambient air quality standards exist or are proposed, provides the data for registration of new pesticides or proposed suspension of those already in use, conducts research on hazardous and toxic materials, and is preparing the health basis for non-ionizing radiation standards. Direct support to the regulatory function of the Agency is provided in the form of expert testimony and preparation of affidavits as well as expert advice to the Administrator to assure the adequacy of health care and surveillance of persons having suffered imminent and substantial endangerment of their health.

The human population is constantly exposed to low levels of many different types of chemicals in the environment. Experimental investigations have emphasized the testing of individual chemical compounds for their oncogenic potential, but relatively little is known about the oncogenic potential of the various chemicals in combination exposures in the environment. The present investigation, therefore, is important since it is designed to learn more about the oncogenic potential associated with combined environmental exposure to different chemicals. Specifically, it is concerned with the interaction between commonly used pesticides and benzo(a)pyrene, a known oncogenic agent.

F. G. Hueter, Ph. D.
Acting Director,
Health Effects Research Laboratory

ABSTRACT

The insecticides parathion, toxaphene and carbaryl were tested for their ability to induce tumors in forestomach and lung of female Ha/ICR and A/J mice, respectively. None of these prototype insecticides, when fed alone in the diet of mice, showed significant tumorigenic activity.

Results of our research show that increased benzo(a)pyrene (BP) hydroxylase activity generally enhances tumor formation while a decrease in BP hydroxylase activity has a protective effect against tumors. For example, when BP was administered chronically or acutely to Ha/ICR mice, forestomach showed the greatest inducibility of BP hydroxylase while it was also the site of BP-induced tumors. Papillomatous tumors developed in the forestomach of mice fed BP for 12 weeks, but no tumors developed in the glandular portion of stomach, or in the lung or liver.

Various dosage levels of insecticides were investigated as to their ability to alter tissue BP hydroxylase activity. In the same Ha/ICR strain of mice, the organochlorine insecticide toxaphene enhanced BP-induced forestomach tumors and increased enzyme activity in forestomach. Also, the carbamate insecticide carbaryl, when fed in the diet for 20 weeks, was found to increase BP hydroxylase activity in lung while enhancing BP-induced tumors in that organ of female A/J mice. These results are discussed in connection with the hypothesis that toxaphene and carbaryl have a cooncogenic effect in enhancing BP-induced tumors. Conversely, when toxaphene inhibited the BP hydroxylase activity in lung there was an associated decrease in BP-induced tumors in A/J mice. Feeding mice the organophosphate insecticide parathion, did not affect tissue BP hydroxylase activity or the incidence of tumors induced by the administration of BP.

The relationship between enzyme inducibility and tumor formation may be due to the level of oncogenic epoxides formed at target organs. As the enzyme levels increase, more epoxide may be formed. Conversely, as enzyme activity decreases, less epoxide is formed. Enzyme inducibility does not always produce tumors, as evidenced by the finding that liver BP hydroxylase was induced after toxaphene feeding but no tumors were observed at this site after treatment with insecticide and/or BP. Further studies of the formation of specific oncogenic epoxides of BP in tissues after treatment with these insecticides would more clearly define the relationships between BP hydroxylase inducibility and BP oncogenesis.

CONTENTS

Disclaimer	ii
Foreword	iii
Abstract	iv
Abbreviations and Symbols	vi
1. Introduction	1
2. Material and Methods	3
Chemicals	3
Tissue BP Hydroxylase Activity	3
Treatment of Animals	4
Statistical Analysis	4
3. Results and Discussion	5
4. Tables	13
References	28

LIST OF ABBREVIATIONS AND SYMBOLS

ABBREVIATIONS

NADPH	-- β-Nicotinamide adenine dinucleotide phosphate, reduced form
NADH	-- β-Nicotinamide adenine dinucleotide, reduced form
min	-- minute
po	-- orally
ppm	-- parts per million
wk	-- wk
SD	-- standard deviation

SYMBOLS

BP	-- benzo(a)pyrene
ml	-- milliliter
mg	-- milligram
g	-- gram
μ	-- micro
n	-- nano
p	-- pico
Km	-- Michaelis constant
M	-- Molar
%	-- percent

SECTION I

INTRODUCTION

The human population is exposed chronically to low levels of insecticides and benzo(a)pyrene (BP). The latter agent is generally acknowledged to be a carcinogenic polycyclic hydrocarbon present throughout our environment. BP is present in tobacco smoke, charcoal grilled foods and as an atmospheric pollutant emitted into air from the general industrial combustion of fuels and organic matter (1,2,3). In addition, studies have shown that in certain strains of mice (4,5) the organochlorine insecticides,DDT, aldrin and dieldrin are hepatic carcinogens. Traces of insecticides are also commonly found in food, and like BP, human exposure could occur from inhalation of tobacco smoke (6). Since man is exposed chronically to the well-known carcinogen BP as well as to insecticides, it is important to know more about the oncogenic potentials associated with combined environmental exposure to these two types of chemicals.

The metabolism of BP is mediated by the microsomal mixed function oxidase enzyme system. In this system, BP hydroxylase has been shown to be the enzyme which activates BP to form strong electrophilic metabolites (7,8). These metabolites possess the ability to bind with nucleophilic groups of nucleic acids and other macromolecules of target organs (9,10). The mutagenic activity of a large number of BP derivatives suggest that highly reactive epoxide metabolites (e.g. Diol-epoxide I and Diol-epoxide II) might be the ultimate carcinogens of BP (11,12,13, 14). Recently it has been reported that the administration of BP7,8-dihydrodiol, a metabolite of BP, to newborn mice caused more malignant lymphomas and pulmonary adenomas than that induced by BP (15).

Various insecticides have been reported to alter the activity of enzymes of the microsomal mixed function oxidase system in different tissues. Parathion, an organophosphate insecticide, inhibits the hepatic microsomal metabolism of aniline and ethylmorphine in mice (16). Studies from our laboratory show that parathion inhibits the metabolism of BP in liver, lung and small intestine in rats (17). Furthermore, experiments with the organochlorine insecticide toxaphene, show that feeding various levels of this insecticide to rats increased the activity of hepatic enzymes that metabolize EPN, p-nitroanisole and aminopyrine (18). Similar effects on hepatic microsomal enzyme induction were observed with the carbamate insecticide carbaryl. Subacute administration of carbaryl produces an increase in the rate of metabolism of hexobarbital, aniline and benzphetamine in mice (19,16).

Few reports have appeared in the literature regarding the possible carcinogenic and/or cocarcinogenic action of these widely used and chemically different insecticides such as parathion, toxaphene and carbaryl. A recommendation by the National Cancer Advisory Board Subcommittee on Environmental Carcinogenesis suggests that one criterion which may be used for assessing whether chemicals are potentially carcinogenic could include "Bioassays in which, in addition to the test agent, animals are treated with a known carcinogen, or some other foreign material which itself may be carcinogenic or cocarcinogenic" (20). Since insecticides alter microsomal enzyme systems which may be responsible for the metabolism of BP to an active carcinogen, we investigated the effects of 3 different chemical prototype insecticides (parathion, toxaphene and carbaryl) on the oncogenic action of BP. The influence of these insecticides on BP hydroxylase activity in various tissues was also determined in order to help elucidate possible mechanisms that may be involved with changes observed in BP-induced oncogenesis. The results of this study could be of value for the determination of potential human health hazards associated with exposure to commonly used insecticides alone and, perhaps more importantly, in the presence of the carcinogenic polycyclic aromatic hydrocarbons such as BP, which is present in the environment.

SECTION 2

MATERIALS AND METHODS

Chemicals - BP (practical grade, minimum purity, 95%) Tris buffer and the cofactors NADH and NADPH were obtained from Sigma Chemical Co. (St. Louis, Mo.). Parathion (95% purity), 0,0-diethyl-0-(p-nitrophenyl) phosphorothioate was purchased from K and K Laboratories (Jamaica, N.Y.). Carbaryl (technical grade, 100% purity) was donated by the Union Carbide Corp. (Salinas, Ca.). Toxaphene (technical grade) was donated by Hercules Incorporated (Wilmington, De.). All control and experimental diets containing BP and/or insecticides were prepared by Bio-Serv, Inc. (Frenchtown, N.J.).

Tissue BP hydroxylase activity - Mice were sacrificed after chronic feeding ad libitum of the various levels of parathion, toxaphene, carbaryl and BP. All homogenates were made in ice-cold 1.15% potassium chloride solution for assay of BP hydroxylase activity by the method of Nebert and Gelboin (21). The final incubation mixture had a volume of 1.05 ml which was composed of: 0.3 ml of NADPH (0.5 umoles), 0.3 ml of NADH (0.5 umoles), 0.2 ml of 0.5 M Tris buffer at pH 7.5, 0.2 ml of a 900 x g supernatant of tissue homogenates containing between 2.3 and 5.5 mg protein and 0.05 ml of a methanol solution of BP substrate (80 nmoles), which was added just prior to incubation. Values for BP hydroxylase activity represent the amount (in pmoles) of reference standard 3-hydroxybenzo(a)pyrene (provided by Dr. Harry V. Gelboin, National Cancer Institute, Bethesda, Md.) causing fluorescence equivalent to the total hydroxylated metabolites produced per milligram protein per min of incubation. Aliquots of the 900 x gravity supernatant from each tissue homogenate were used for protein determination by the method of Lowry et al. (22). When assaying for BP hydroxylase activity in forestomach and glandular stomach, stomach tissue was dissected into 2 parts. The pooled forestomach of 3 animals were used to prepare homogenates. Under the conditions of the assay, the rate of hydroxylation of BP was linear for 60 min for both forestomach and glandular stomach from untreated control mice. The Michaelis constant (Km) determined at a 30 min period of incubation was 1.20×10^{-5}M for forestomach and 1.09×10^{-5}M for glandular stomach.

Treatment of animals - All experiments were conducted with female Ha/ICR mice purchased at 8 weeks of age from ARS/Sprague Dawley (Madison, Wisconsin) or female A/J mice purchased at 8 weeks of age from the Jackson Laboratory (Bar Harbor, Maine). After one week of acclimation to the quarters, the animals were randomized and placed on either diets of Purina Rat Chow to which had been added 5% corn oil (control group), or experimental diet containing 5% corn oil as the vehicle and various levels of insecticides and/or BP. In studies involving Ha/ICR mice, BP was incorporated into the diet. The A/J mice, however, were administered 3 mg BP (po) on the 7th and 21st day of the experiment. Animals were housed in suspended wire mesh cages, five to a cage, and weighed weekly. After various time periods (12-20 weeks) of feeding, the mice were sacrificed for tumor analysis and/or enzyme activity. The presence of stomach tumors in all experiments was determined by expanding the stomachs with an intragastric injection of a 10% buffered formalin solution. Within 2-3 days the stomachs were opened, and observed grossly for tumor counts under a dissecting microscope as described by Wattenberg (23). Lungs were placed in Tellyesniczky's acetic bichromate solution for 2 days and then examined grossly. In addition, liver and other internal organs were observed grossly for possible tumor formation. Tumors measuring 1.0 mm or larger were counted, recorded, fixed in buffered formalin and embedded in paraffin. They were sectioned and stained with hematoxylin-eosin by the standard procedures, and assessed microscopically for tumor type. The relative susceptibility to BP-induced tumors is expressed by the "tumorigenic index", derived by multiplying the percentage of animals in which tumors develop by the average number of tumors per tumor-bearing animal as proposed by Shimkin (24).

Statistical Analysis. All data shown for weight gain and BP hydroxylase activity in this study were analyzed statistically by Student's t-test. For the analysis of tumor incidence between the control and experimental groups the "non-parametric" rank t-test of Bross (25) or Fisher's exact test were used. Only probability values of 0.05 or less were considered significant.

SECTION 3

RESULTS AND DISCUSSION

The effect of feeding two dosage levels of BP in the diet of Ha/ICR mice for 12 or 20 weeks on the development of forestomach tumors is shown in Table 1. The Ha/ICR mouse was used in this study because of its susceptibility to forestomach tumors after exposure to polycyclic hydrocarbons such as BP. The incidence of tumor formation expressed as the carcinogenic index is shown to be dose-dependent for the 12 week feeding period. More than one tumor was found in each tumor-bearing animal, and microscopic examination of forestomach revealed that these tumors were of the squamous papilloma type. After 20 weeks of BP feeding, all of the mice in the 200 ppm dietary group were shown to have developed tumors, and in animals fed higher dietary levels of BP (300 ppm), the tumors could not be accurately counted because the entire lumen of the forestomach was occluded by the formation of well-developed squamous cell papillomas. No tumors were found in the forestomach of the respective control mice. In addition, gross examination of glandular stomach, lung and liver from control mice and those fed BP were also found to be negative for the presence of tumors.

This report shows that the forestomach is susceptible to the development of tumors after a 12-week feeding period of BP in the diet of mice. Glandular stomach did not form tumors, thus appearing to be resistant to the carcinogenic action of BP. The mechanism by which squamous cells of the forestomach give rise to papillomatous tumors by feeding BP is not known. Selectivity for tumor formation in forestomach has been previously reported for BP in different strains of mice (23,26). Previous studies (27,28,29,30) using different strains of mice have shown a correlation between induction of BP hydroxylase activity and the formation of tumors in various target organs by the polycyclic hydrocarbon, 3-methylcholanthrene. Studies on DNA binding (10) and mutagenic activity (11,12,13,14) in vitro of a large number of BP derivatives suggest that the most active carcinogenic metabolite of BP is the 7β, 8α-dihydroxy-9α, 10α-epoxy-7,8,9,10-tetrahydrobenzo(a)pyrene. Since the BP hydroxylase enzyme system

is thought to be essential for the metabolic activation of BP to form this reactive epoxide, it is important to know whether the basal level and/or the inducible level of BP hydroxylase activity in tissues is associated with tumor formation by the administration of BP.

The induction of BP hydroxylase activity at the site of tumor formation in forestomach, and in other tissues, is presented in Table 2. The basal level of BP hydroxylase in control forestomach (0.212±0.03) was significantly higher than in the glandular stomach (0.060±0.01); $p < 0.01$. After the mice were fed for 12 weeks with BP, the BP hydroxylase activity was increased by 4.2- to 5.2-fold in forestomach, whereas induction of BP hydroxylase was lower (2.2-3.9-fold) in glandular stomach. In lung, the BP hydroxylase activity was significantly increased by 1.6-fold only at the higher dietary level of BP. However, in liver where the basal level of BP hydroxylase activity was found to be highest of all tissues studied, chronic feeding of BP did not cause induction of BP hydroxylase activity. BP hydroxylase activity was also determined in these same tissues and in small intestine and kidney, 24 hr after a single p.o. dose (20 mg/kg) of BP (Table 3). Under these experimental conditions, BP was found to increase significantly this enzyme system in forestomach, glandular stomach, small intestine, and lung, but kidney and liver BP hydroxylase was not significantly changed. Similar to our previous results with the chronic feeding of BP presented in Table 2, after a single acute dose of BP, BP hydroxylase activity is induced to the greatest extent in forestomach (4.3-fold); this is followed by glandular stomach (2.4-fold), small intestine (2.3-fold), and lung (1.9-fold). The basal level in control mice of BP hydroxylase activity was again significantly higher in forestomach than in glandular stomach ($P < 0.01$).

The results presented in Tables 2 and 3 of this report show that the basal level of BP hydroxylase was higher in forestomach, a site sensitive to tumor formation, than in glandular stomach. Since no tumors were formed in tissues with higher basal enzyme activity than forestomach, such as in lung, small intestine, or liver, it seems unlikely that the basal level of BP hydroxylase is the major factor in the tumorigenic effects of BP.

It was further shown in this report that, in all tissues examined, the induction of BP hydroxylase activity was highest in forestomach after either acute p.o. administration or chronic dietary feeding of BP. Data similar to that reported in this paper on induction of BP hydroxylase activity in various areas of rat stomach were reported by Wattenberg *et al.* (31). They

found that rat forestomach had moderate basal BP hydroxylase, whereas, in the glandular portion, it was barely detectable. After p.o. administration of 1,2-benzanthracene, forestomach was approximately 5 times more active in enzyme activity than glandular stomach. In our experiments with mice, the BP hydroxylase activity was also approximately 3 to 5 times higher in forestomach than glandular stomach after the administration of BP.

It is generally believed that BP must be metabolically activated by the BP hydroxylase enzyme to form reactive carcinogenic epoxides. Our findings in Ha/ICR mice of tumor formation only in the forestomach associated with the highest level of BP hydroxylase inducibility at this site following the administration of BP supports the hypothesis previously proposed by other investigators (27,28,29,30,32) that induction of BP hydroxylase is related to the formation of tumors following the administration of various carcinogenic polycyclic hydrocarbons. An explanation as to why BP hydroxylase activity was also inducible in glandular stomach, small intestine and lung, sites that had been found to be resistant to tumor formation by the administration of BP, is not available at the present time. Further studies comparing forestomach with other tissues having inducible AHH activity to form and/or inactivate the various tumorigenic epoxides formed from BP may resolve the question as to why the forestomach develops tumors and why other tissues with inducible AHH activity do not develop tumors.

Studies on the effects of various levels of insecticides in the diet on weight gain and forestomach tumor formation in Ha/ICR mice can be seen in Table 4. In the case of the organophosphate insecticide parathion two experiments were carried out. In the first experiment, levels of 100 and 150 ppm of parathion were found to inhibit significantly weight gain after 12 weeks of feeding. In the tumorigenic testing of chemicals, factors such as body weight changes are extremely important, for it is well known that all other factors being equal, a decreased gain in body weight reduces the occurrence of tumors (33,34). Since the mice could not tolerate these high levels of parathion in the diet, the experiment was repeated using a lower dose of 75 ppm. Animals fed the lower dietary level of parathion (75 ppm), the organochlorine insecticide toxaphene (100 to 400 ppm), or the carbamate insecticide carbaryl (500 to 2000 ppm) showed no significant difference in weight gain from their respective controls after 12 weeks. Examination of forestomachs from the control, parathion, toxaphene and carbaryl groups of mice revealed no tumors grossly or microscopically. In addition, the glandular stomach, liver, and lung from the controls and all experimental groups showed no evidence of tumor formation when examined grossly.

The data on tissue BP hydroxylase activity from mice exposed to various levels of the three insecticides are presented in Table 5. Parathion at a dosage level of 75 ppm showed no change in BP hydroxylase activity in the liver and stomach; however, enzyme activity was significantly decreased in the lung.

Toxaphene, fed at a level of 100 to 400 ppm, produced increases in liver BP hydroxylase activity, and was found to decrease lung BP hydroxylase activity. No significant change in enzyme activity in the stomach was observed for any of the dietary levels of toxaphene tested. Furthermore, the data in Table 5 show that carbaryl feeding did not affect BP hydroxylase activity in liver and lung, but significantly decreased activity in stomach at the highest dietary level (2000 ppm) tested. These results on the inducibility of tissue BP hydroxylase activity after insecticides are in agreement with the work previously reported by others (35,36) which showed that enzyme induction after the administration of various polycyclic hydrocarbons varies from tissue to tissue in a given species or strain of animal.

Studies carried out on the effects of these insecticides on BP-induced tumors in the forestomach of Ha/ICR mice are presented in Table 6. A feeding level of 200 ppm BP was chosen for these experiments based on our previous data (Table 1) showing that this level of BP resulted in a tumor incidence of 67% of the test animals, and did not affect weight gain during a 12-week feeding period. The data show that neither parathion, nor carbaryl affects the tumorigenic action of BP in forestomach. When toxaphene was tested for its effects on BP-induced tumorigenesis, 60% of the mice fed 200 ppm BP had squamous papillomas of the forestomach. In mice fed a combination of 400 ppm toxaphene and 200 ppm BP, 87% had similar gastric tumors. Toxaphene was found to increase the mean number of tumors per mouse, and the Bross (25) rank t-test comparison revealed a statistically significant difference between groups. It should also be noted from the data presented in Table 4, that the weight gain in the experimental group fed toxaphene and BP was significantly less than in the control group fed BP only. Though nutritional factors tional factors are known to affect tumor incidences and might account for the observed increase of tumors, other investigators have reported that decreased body weight gain due to a restricted caloric intake results in a decreased incidence of tumors induced by carcinogenic hydrocarbons in various target organs of mice (33,34). Therefore, the increased incidence of tumors found after feeding both toxaphene and BP does not appear to be related to the observed decreased growth rate in this group.

A possible explanation for the increased incidence of BP-induced forestomach tumors in Ha/ICR mice after exposure to the organochlorine insecticide toxaphene, is that toxaphene itself may be oncogenic. A study by the National Cancer Institute reported that mice fed toxaphene for 22 months developed an increased incidence of tumors in liver (unpublished data, 37). Under the conditions of the present experiments, the shorter time period (12 weeks) of feeding toxaphene may be inadequate to demonstrate an oncogenic effect. However, a suboncogenic effect by toxaphene may be synergistic for the development of forestomach tumors in mice fed the carcinogen BP in combination with toxaphene. Some early experiments with male rats (38) show that simultaneous feeding of the carcinogens 2-acetylaminofluorene and 3'-methyl-4-dimethylaminoazobenzene resulted in a

synergistic effect for the development of liver tumors.

Another more likely explanation for the increase in BP-induced tumors found in forestomach is that toxaphene may act as a cocarcinogen as defined by Berenblum (39). The type of cocarcinogenic influence that toxaphene may be exerting is "a permissive influence" affecting the metabolism of the oncogen prior to its action. Detailed studies have shown that the microsomal mixed function oxidase system has the capacity both to activate chemicals such as BP to ultimate carcinogenic forms and to detoxify them to inactive products (9,10,11,12,13,14). BP hydroxylase is thought to be essential for the metabolism of BP to form reactive carcinogenic epoxides. Before we can explain any possible interactions between insecticides and BP, it is important to know whether these insecticides alter BP hydroxylase activity, especially in target organs.

Since toxaphene was found to enhance BP-induced tumors in forestomach, but not affect BP hydroxylase activity in whole stomach, a short term (2 weeks) feeding experiment was conducted to determine the effects of the various chemical prototype insecticides on BP hydroxylase activity in forestomach, a site specific for induction of tumors by BP. For comparative purposes enzyme activity was also measured in the glandular portion of stomach. The results of this study are shown in Table 7. After feeding mice for only 2 weeks with these insecticides, BP hydroxylase activity was significantly increased in the forestomach by parathion and toxaphene. No change was observed in forestomach BP hydroxylase activity after the feeding of carbaryl. In the glandular portion of the stomach, which is resistant to BP carcinogenesis, there was no effect by the insecticides on BP hydroxylase activity. From this data, we see that toxaphene acts as an inducer of BP hydroxylase activity in that portion of the stomach (forestomach) which is susceptible to the carcinogenic action of BP.

Similar studies on the interaction of insecticides and BP were conducted on A/J mice, a strain with a high spontaneous rate of lung tumors. A pilot study utilizing a small number of A/J mice revealed that their weight gain was inhibited significantly when the three prototype insecticides (parathion, toxaphene and carbaryl) were fed at the maximum tolerated doses previously established for the Ha/ICR strain of mice. For this reason, it was necessary to reduce the dosage level of each insecticide in the diet for further studies with the A/J strain of mice. Data concerning the effects of feeding parathion in the diet for 20 weeks on lung tumors are found in Table 8. The data show that although mice treated with parathion had a higher percentage of tumors than controls (43% vs. 13%), this difference was not statistically significant. Because these results were inconclusive, a second experiment was conducted using larger groups of mice. The results with regard to the gross examination of lung lesions found after parathion administration appeared at first to be similar to those of the first experiment and are presented at the bottom of Table 8. In this

second experiment, however, careful microscopic examination revealed that most of the lung lesions in the parathion group were probably inflammatory rather than neoplastic in nature, while those in the control group were considered to be neoplastic. Thus the lesions seen grossly in the parathion group were more likely due to infection rather than oncogenesis.

Data pertaining to the effect of parathion on BP-induced tumors may be found in Table 9. Since BP treatment in the first experiment caused all of the animals to develop lung tumors after 20 weeks, a possible enhancing effect by parathion feeding on BP-induced tumors could not be demonstrated. Therefore, we repeated the experiment for a shorter time period (16 weeks) in an attempt to cause a BP-induced tumor incidence of approximately 50%. Table 9 shows no difference in the incidence of BP-induced tumors after parathion feeding for 16 weeks when compared to the control group. Microscopic examination of tumors induced by BP alone for the data presented in Table 9 indicated that 70% of the lung tumors induced were alveolar, while 30% were of the bronchiolar type. In the group administered parathion and BP, approximately 95% of the lung tumors were alveolar and the remaining 5% were bronchiolar.

Data relating to BP hydroxylase are shown in Table 10. The results indicate that parathion fed in the diet for 20 weeks and/or administration of BP orally did not affect BP hydroxylase activity in liver, lung or forestomach.

Studies to determine whether the organochlorine insecticide toxaphene affects the incidence of lung tumors, or affects BP-induced tumors were carried out, and the data are presented in Table 11. While toxaphene alone failed to affect the tumor incidence in the lung of the A/J mouse, it was found to profoundly decrease the formation of BP-induced lung tumors in this strain of mice. Toxaphene decreased the percentage and the mean number of tumors per mouse induced by BP, and a rank t-test comparison of the lung tumor incidence revealed that there was a statistically significant difference between groups. Microscopic examination of the lung from animals treated with toxaphene and/or BP revealed that in mice given BP alone, 76% of the lung tumors were the alveolar type and 23% were bronchiolar. In mice administered toxaphene and BP, 33% were alveolar and 67% were bronchiolar. No inflammatory lesions were observed in either of these groups. The data pertaining to BP hydroxylase activity after toxaphene alone and in combination with BP are presented in Table 12. By feeding 200 ppm of toxaphene enzyme activity was induced in liver but not affected in forestomach. More importantly, in lung where toxaphene feeding was found to markedly decrease BP-induced tumors, there was a significant decrease in BP hydroxylase. Thus, in the A/J strain of mice toxaphene exerts a protective effect against BP-induced tumors. This effect of toxaphene is associated with a corresponding decrease in BP hydroxylase activity in lung as the target organ. Other studies showing that inhibition of BP hydroxylase activity protects against tumorigenesis have been reported by Gelboin *et al.* (40). These investigators showed that 7,8-benzoflavone, when added to homogenates of skin epidermis *in vitro*, inhibits BP hydroxylase activity. When

applied topically, 7,8-benzoflavone inhibits the formation of mouse skin tumors after repeated treatment with 9,10-dimethylbenzanthracene.

The results presented in Table 12 on enzyme activity for toxaphene are similar to those observed for 7,8-benzoflavone on BP hydroxylase activity in different tissues of rats as reported by Weibel et al. (41). These investigators found a decrease in the lung, but an increase in liver BP hydroxylase activity when these tissues were incubated with 7,8-benzoflavone in vitro. They concluded that at least two forms of the BP hydroxylase enzyme complex exist. One form predominates in hepatic tissue and the other predominates in extra-hepatic tissues such as the lung. This may partly explain why toxaphene does not cause tumor formation in liver, although BP hydroxylase is induced by this insecticide in this tissue. It may be that liver has a different cytochrome P-450, which forms metabolites of BP that do not covalently bind DNA, RNA or other macromolecules which in turn initiate the carcinogenic process in this organ.

As shown in Table 13, the data with regard to carbaryl feeding were similar to that observed for parathion. Mice that were fed carbaryl alone had more tumors than the control mice but the difference was not statistically significant. As with the parathion study, we repeated the original carbaryl study using larger numbers of animals in order to obtain more conclusive results. Data for the second experiment are presented at the bottom of Table 13 and show no significant difference in gross lung tumor incidence after carbaryl feeding. This indicates that the previous results, which cast suspicion on carbaryl as being tumorigenic, were probably due to chance. In contrast to the parathion interaction study with BP, carbaryl feeding for 20 weeks was found to enhance BP-induced tumors in lung (Table 14). Since the tumor incidence for mice given BP alone was close to 100% after 20 weeks, the experiment was repeated for 16 weeks, a lesser time period, in an effort to decrease BP-induced tumor incidence to approximately 50%. The data from this second experiment in Table 14 show that carbaryl did not affect the incidence of BP-induced lung tumors after 16 weeks. The difference in the results of the two experiments could be due to the different time periods. Perhaps a longer time period is necessary for carbaryl to have an effect. Further studies could be done utilizing a longer time period (i.e. 24 weeks) and a lower dose of BP than we used which would yield approximately a 50% tumor incidence. After microscopic examination of lung tissue, 84% of the lung tumors induced by BP alone were found to be alveolar and 16% were bronchiolar. In mice given BP and carbaryl, 70% of the lung tumors were alveolar, while 30% were bronchiolar. No inflammatory lesions were found in the lungs of these animals. The data found in Table 15 show that lung BP hydroxylase was significantly higher in mice fed carbaryl for 20 weeks and given BP orally than in those administered BP only. As in previous data of this report, this indicates an association exists between induction of BP hydroxylase and enhancement

of BP-induced tumors. Data in Table 15 show carbaryl slightly inhibited enzyme activity in forestomach, but this insecticide did not affect liver BP hydroxylase activity.

Evidence from our research shows that increased BP hydroxylase generally enhances tumor formation while a decrease in BP hydroxylase has a protective effect against tumors. The relationship between enzyme inducibility and tumor formation may be due to the level of tumorigenic epoxides formed at target organs. As the enzyme levels increase, more epoxide may be formed, and conversely, as enzyme activity decreases, less epoxide is formed. Enzyme inducibility, however, does not always produce tumors. Additional research on these insecticides is needed to study the binding of the epoxides to the DNA, RNA and macromolecules which initiate the tumorigenic process so that the whole mechanism for tumor enhancement may be understood.

TABLE 1. EFFECT OF VARIOUS LEVELS OF BP IN THE DIET ON BODY WEIGHT GAIN AND TUMOR FORMATION IN FORESTOMACH OF Ha/ICR MICE

Mice were fed a diet to which had been added 5% corn oil (control) or 5% corn oil with 2 dosage levels of BP in the diet. Mice were 9 weeks old at the start of the experiment and, after 12 or 20 weeks on the diet, were sacrificed for tumor count.

Experimental Group	Amount of Carcinogen added to Diet (ppm)	Duration of Experiment (wk)	Number of Mice	Weight Gain* (g)	Mice with Tumors (%)	Number of Tumors/ Mouse †	Tumorigenic Index ‡
Control	0	12	14	8.3	0	0.0	0
BP	200	12	15	7.2	67	1.8±0.7	121
BP	300	12	14	6.7	100	4.0±2.1	400
Control	0	20	5	11.5	0	0.0±0.0	0
BP	200	20	6	11.2	100	4.8±2.2	480
BP	300	20	5	8.5	100	#	---

*Mean weight gain per group during interval between start of experimental diets and time mice were killed.

† Mean ± SD calculated for only tumor-bearing mice.

‡ Percentage of mice with tumors times the mean number of tumors per tumor-bearing mouse.

Tumor count not available due to the tumors forming a large mass in the forestomach.

TABLE 2. EFFECT OF VARIOUS LEVELS OF BP IN THE DIET ON TISSUE BP HYDROXYLASE ACTIVITY OF Ha/ICR MICE

Mice were fed a diet to which had been added 5% corn oil (control) or 5% corn oil with 2 dosage levels of BP in the diet. Mice were 9 weeks old at the start of the experiment and, after 12 weeks, were sacrificed for enzyme assay. The results are expressed as pmoles of 3-hydroxybenzo(a) pyrene per mg of protein per min. Each value represents the mean ± S.D. of 4 determinations.

Experimental Group	Amount of Carcinogen Added to Diet (ppm)	BP Hydroxylase Activity							
		Forestomach	Ratio*	Glandular stomach	Ratio	Lung	Ratio	Liver	Ratio
Control	0	0.212±0.03		0.060±0.01		2.95±0.74		60.97± 9.91	
BP	200	0.833±0.28†	4.2	0.131±0.01†	2.2	3.69±0.49	1.3	50.97± 7.00	0.8
BP	300	1.100±0.43†	5.2	0.228±0.09†	3.9	4.57±0.88‡	1.6	55.62±17.49	0.9

*Ratio, BP hydroxylase activity of mice receiving BP divided by the activity of the control group

†Significantly different from control group ($P < 0.01$)

‡Significantly different from control group ($P < 0.05$)

TABLE 3. ACUTE EFFECTS OF BP ON TISSUE BP HYDROXYLASE ACTIVITY OF Ha/ICR MICE

Mice were given BP (20 mg/kg) p.o. in a 10% solution of ethanol in corn oil of 1% of body weight 24 hr prior to sacrifice for measurement of enzyme activity. The corresponding controls were given an equivalent amount of vehicle. The results are expressed as pmoles of 3-hydroxybenzo(a)pyrene per mg of protein per min. Each value represents the mean ± S.D. of 3 to 4 determinations.

Experimental Group	Amount of Carcinogen Given Orally (mg/kg)	BP Hydroxylase Activity					
		Forestomach	Glandular stomach	Small Intestine	Lung	Kidney	Liver
Control	0	0.241±0.06	0.103±0.02	8.49±4.02	3.81±1.36	0.297±0.08	45.27±4.38
BP	20	1.037±0.18*	0.251±0.09†	19.48±4.37†	7.31±0.77‡	0.463±0.17	53.45±6.35
	Ratio#	4.3	2.4	2.3	1.9	1.6	1.2

*Significantly different from control group ($P < 0.001$)

†Significantly different from control group ($P < 0.05$)

‡Significantly different from control group ($P < 0.01$)

#Ratio BP hydroxylase activity of mice receiving BP divided by the activity of the control group

TABLE 4. EFFECT OF VARIOUS LEVELS OF PARATHION, TOXAPHENE AND CARBARYL IN THE DIET ON BODY WEIGHT GAIN AND TUMOR FORMATION IN THE FORESTOMACH OF Ha/ICR MICF

Mice were fed a diet to which had been added 5% corn oil (control) or 5% corn oil with various dosage levels of insecticide. Mice were 9 weeks old at the start of the experiment and, after 12 weeks on the diet, were sacrificed for tumor count.

Experimental Group	Amount of Insecticide Added to Diet (ppm)	Number of Mice	Weight Gain* (g)	Mice with Tumors
Control	0	15	10.1	-
Parathion	100	15	6.8†	-
Parathion	150	15	6.9†	-
Control	0	15	9.5	0
Parathion	75	16	8.3	0
Control	0	15	9.0	0
Toxaphene	100	14	6.8	0
Toxaphene	200	15	6.8	0
Toxaphene	400	15	7.2	0
Control	0	15	7.2	0
Carbaryl	500	15	8.4	0
Carbaryl	1000	14	6.1	0
Carbaryl	2000	15	5.4	0

*Mean weight gain per group during interval between start of experimental diets and time mice were killed

†Significantly different from control group ($P < 0.01$)

TABLE 5. EFFECT OF VARIOUS LEVELS OF PARATHION, TOXAPHENE AND CARBARYL IN THE DIET ON TISSUE BP HYDROXYLASE ACTIVITY OF Ha/ICR MICE

Mice were fed a diet to which had been added 5% corn oil (control) or 5% corn oil with various dosage levels of insecticide. Mice were 9 weeks old at the start of the experiment and, after 12 weeks, were sacrificed for enzyme assay. The results are expressed as pmoles of 3-hydroxybenzo(a)pyrene per mg of protein per min. Each value represents the mean ± S.D. of 4 or 5 determinations.

Experimental Group	Amount of Insecticide Added to Diet (ppm)	BP Hydroxylase Activity		
		Liver	Lung	Stomach
Control	0	50.7±10.8	5.42±0.65	0.158±.092
Parathion	75	46.4± 5.4	3.40±0.44‡	0.195±.074
Control	0	66.8±11.4	2.43±0.30	0.166±.018
Toxaphene	100	103.1± 8.7‡	0.54±0.18‡	0.195±.069
Toxaphene	200	102.2±10.4‡	0.33±0.10‡	0.302±.211
Toxaphene	400	99.3±14.9†	0.36±0.18‡	0.232±.103
Control	0	54.9± 6.7	4.32±1.54	0.169±.035
Carbaryl	500	56.9± 3.8	3.76±1.18	0.144±.024
Carbaryl	1000	61.5± 6.9	3.47±0.81	0.206±.140
Carbaryl	2000	61.5±11.5	3.39±0.38	0.110±.042*

*Significantly different from control group ($P < 0.05$)

†Significantly different from control group ($P < 0.005$)

‡Significantly different from control group ($P < 0.001$)

TABLE 6. EFFECT OF PARATHION, TOXAPHENE AND CARBARYL IN THE DIET ON BODY WEIGHT GAIN AND BP-INDUCED TUMOR FORMATION IN THE FORESTOMACH OF Ha/ICR MICE

Mice were fed a diet to which had been added 5% corn oil (control) and BP, or 5% corn oil, insecticide and BP. Mice were 9 weeks old at the start of the experiment and, after 12 weeks on the diet, were sacrificed for tumor count.

Experimental group							
Amount of Insecticide Added to Diet (ppm)	Amount of Carcinogen Added to Diet (ppm)	Duration of Experiment (wk)	Number of Mice	Weight Gain* (g)	Mice with Tumors (%)	Number of Tumors/ Mouse†	Tumorigenic Index‡
Control	BP 200	12	13	4.3	53	2.5±0.5	133
Parathion 75	BP 200	12	13	5.2	53	2.1±1.3	111
Control	BP 200	12	15	7.9	60	1.9±1.2	114
Toxaphene 400	BP 200	12	15	4.5§	87#	2.5±1.2	217
Control	BP 200	12	14	7.9	43	1.7±0.8	73
Carbaryl 2000	BP 200	12	14	7.5	43	2.3±1.9	99

*Mean weight gain per group during interval between start of experimental diets and time mice were killed

†Mean ± SD calculated from tumor-bearing mice

‡Percentage of mice with tumors times the mean number of tumor per tumor-bearing mouse

#Significantly different from control group ($P < 0.05$)

§Significantly different from control group ($P < 0.01$)

TABLE 7. EFFECT OF PARATHION, TOXAPHENE AND CARBARYL IN THE DIET ON TISSUE BP HYDROXYLASE ACTIVITY OF Ha/ICR MICE

Mice were fed a diet to which had been added 5% corn oil (control) or 5% corn oil with an insecticide. Mice were 9 weeks old at the start of the experiment and, after 2 weeks, were sacrificed for enzyme assay. The results are expressed as pmoles of 3-hydroxybenzo(a)pyrene per mg of protein per min. Each value represents the mean ± S.D. of 5 or 6 determinations in each of which the pooled tissue of three mice were used.

Experimental Group	Amount of Insecticide Added to Diet (ppm)	BP Hydroxylase Activity	
		Forestomach	Glandular Stomach
Control	0	0.116±0.008	0.061±0.031
Parathion	75	0.136±0.011*	0.054±0.022
Toxaphene	400	0.145±0.017†	0.064±0.025
Carbaryl	2000	0.114±0.011	0.047±0.026

*Significantly different from control group ($P < 0.02$)

†Significantly different from control group ($P < 0.01$)

TABLE 8. EFFECT OF THE ORGANOPHOSPHATE INSECTICIDE PARATHION IN THE DIET ON WEIGHT GAIN AND TUMORS OF THE LUNG OF A/J MICE

Mice were fed diets containing either: 5% corn oil (control) or 5% corn oil and 50 ppm parathion. Mice were 9 weeks old at start of experiment and after 20 weeks on the diet were sacrificed for gross tumor count.

Experimental Group	Amount of Insecticide Added to Diet (ppm)	Number of Mice	Weight Gain* (g)	Mice with Tumors (%)	Number of Tumors/ Mouse †	Tumorigenic Index ‡
Experiment 1						
Control	0	16	6.8	13	1.0±0.0	13
Parathion	50	14	7.8	43 #	1.0±0.0	43
Experiment 2						
Control	0	32	7.0	19	1.2±0.4	23
Parathion	50	32	7.5	37 $	--	--

*Mean weight gain per group during interval between start of experimental diets and time mice were sacrificed

†Mean ± SD calculated from tumor bearing mice

‡Percentage of mice with tumors times the mean number of tumors per tumor bearing mouse

#No significant difference between control group

$ Upon microscopic examination most of the lesions were found to be inflammatory rather than neoplastic

TABLE 9. INTERACTION BETWEEN THE ORGANOPHOSPHATE INSECTICIDE PARATHION IN THE DIET AND THE CARCINOGEN BP (P.O.) ON GROWTH RATE AND NEOPLASIA OF THE LUNG OF A/J MICE

Mice were fed diets containing either 5% corn oil (control) and 3 mg BP given p.o. on the 7th and 21st day of the experiment or 5% corn oil and 50 ppm parathion and 3 mg BP given p.o. on the 7th and 21st day of the experiment. Mice were 9 weeks old at start of experiment and after 16 or 20 weeks on the diet were sacrificed for gross tumor count.

Experimental Group		Duration of Experiment (wk)	Number of Mice	Weight Gain* (g)	Mice with Tumors (%)	Number of Tumors/ Mouse†	Tumorigenic Index‡
Amount of Insecticide Added to Diet (ppm)	Amount of Carcinogen Given Orally						
Experiment 1							
Control I	BP 3 mg p.o. (2X)	20	15	6.8	100	4.3±2.4	430
Parathion 50	BP 3 mg p.o. (2X)	20	15	7.3	100	3.9±2.1	390
Experiment 2							
Control II	BP 3 mg p.o. (2X)	16	15	7.1	67	2.1±1.0	141
Parathion 50	BP 3 mg p.o. (2X)	16	25	6.4	68	1.8±0.6	122

* Mean weight gain per group during interval between start of experimental diets and time mice were sacrificed

† Mean ± SD calculated from tumor bearing mice

‡ Percent of mice with tumors times the mean number of tumors per tumor bearing mouse

TABLE 10. INTERACTION BETWEEN THE ORGANOPHOSPHATE INSECTICIDE PARATHION IN THE DIET AND THE CARCINOGEN BP ON BP HYDROXYLASE ACTIVITY OF THE LIVER, LUNG AND FORESTOMACH OF A/J MICE

Mice were fed diets containing one of the following: 5% corn oil (control I), 5% corn oil and 50 ppm ppm parathion, 5% corn oil (control II) and 3 mg BP given p.o. on the 7th and 21st day of the experiment, or 5% corn oil and 50 ppm parathion and 3 mg BP given p.o. on the 7th and 21st day of the experiment. Mice were 9 weeks old at start of experiment and after 20 weeks on the diet were sacrificed for enzyme assay. The results are expressed as pmole of 3 hydroxybenzo(a)pyrene per mg of protein per min. Each value represents the mean ± S.D. of 4 determinations.

Experimental Group		BP Hydroxylase Activity		
Amount of Insecticide Added to Diet (ppm)	Amount of Carcinogen Given Orally	Liver	Lung	Forestomach *
Control I	None	42.90±10.74	4.51±0.54	0.139±.136
Parathion 50	None	44.45± 7.68	4.46±0.21	0.124±0.0
Control II	BP 3 mg p.o. (2X)	47.55±14.09	4.65±0.50	0.177±.064
Parathion 50	BP 3 mg p.o. (2X)	45.08± 5.48	5.22±0.68	0.152±.042

* Three forestomachs were pooled per determination

TABLE 11. INTERACTION BETWEEN THE ORGANOCHLORINE INSECTICIDE TOXAPHENE IN THE DIET AND THE CARCINOGEN BP ON WEIGHT GAIN AND TUMORS OF THE LUNG OF A/J MICE

Mice were fed diets containing one of the following: 5% corn oil (control I), 5% corn oil and 200 ppm toxaphene, 5% corn oil (control II) and 3 mg BP given p.o. on the 7th and 21st day of the experiment, or 5% corn oil and 200 ppm toxaphene and 3 mg BP given p.o. on the 7th and 21st day of the experiment. Mice were 9 weeks old at start of experiment and after 20 weeks on the diet were sacrificed for gross tumor count

Experimental Group						
Amount of Insecticide Added to Diet (ppm)	Amount of Carcinogen Given Orally	Number of Mice	Weight Gain* (g)	Mice with Tumors (%)	Number of Tumors/ Mouse†	Tumorigenic Index ‡
Control I	None	17	7.3	23	1.0±0,0	23
Toxaphene 200	None	15	6.7	7#	1.0±0,0	7
Control II	BP 3 mg p.o. (2X)	18	6.2	100	7.2±3.5	720
Toxaphene 200	BP 3 mg p.o. (2X)	15	6.9	67§	1.6±0.8	107

*Mean weight gain per group during interval between start of experiment and time mice were sacrificed

† Mean ± SD calculated from tumor bearing mice

‡ Percentage of mice with tumors times the mean number of tumors per tumor-bearing mouse

NS: no significant difference between control I group

§ Significantly different from control II given BP ($P < 0.001$)

TABLE 12. INTERACTION BETWEEN THE ORGANOCHLORINE INSECTICIDE TOXAPHENE IN THE DIET AND THE CARCINOGEN BP ON BP HYDROXYLASE ACTIVITY OF THE LIVER, LUNG AND FORESTOMACH OF A/J MICE

Mice were fed diets containing one of the following: 5% corn oil (control I), 5% corn oil and 200 ppm toxaphene, 5% corn oil (control II) and 3 mg BP given p.o. on the 7th and 21st day of the experiment, or 5% corn oil and 200 ppm toxaphene and 3 mg BP given p.o. on the 7th and 21st day of the experiment. Mice were 9 weeks old at start of experiment and after 20 weeks on the diet were sacrificed for enzyme assay. The results are expressed as pmole of 3-hydroxybenzo(a)pyrene per mg protein per min. Each value represents the mean ± S.D. of 4 determinations

Experimental Group		BP Hydroxylase Activity		
Amount of Insecticide Added to Diet (ppm)	Amount of Carcinogen Given Orally	Liver	Lung	Forestomach*
Control I	None	26.74± 5.57	2.58±0.60	0.082±0.012
Toxaphene 200	None	49.43±13.78†	0.61±0.16‡	0.101±0.018
Control II	BP 3 mg p.o. (2x)	31.17± 9.98	2.51±0.57	0.075±0.020
Toxaphene 200	BP 3 mg p.o. (2x)	64.23±17.61#	0.85±0.06§	0.075±0.010

* Three forestomachs were pooled per determination
†Significantly different from control I group ($P < 0.025$)
‡Significantly different from control I group ($P < 0.001$)
#Significantly different from control II group ($P < 0.020$)
§Significantly different from control II group ($P < 0.005$)

TABLE 13. EFFECT OF THE CARBAMATE INSECTICIDE CARBARYL IN THE DIET ON WEIGHT GAIN AND TUMORS OF THE LUNG OF A/J MICE

Mice were fed diets containing either 5% corn oil (control) or 5% corn oil and 1000 ppm carbaryl. Mice were 9 weeks old at start of experiment and after 20 weeks on the diet were sacrificed for gross tumor count.

Experimental Group	Amount of Insecticide Added to Diet (ppm)	Number of Mice	Weight Gain* (%)	Mice with Tumors (%)	Number of Tumors/ Mouse†	Tumorigenic Index‡
Experiment 1						
Control	0	11	5.4	9	1±0.0	9
Carbaryl	1000	16	6.0	31 #	1.2±0.4	37
Experiment 2						
Control	0	31	6.3	23	1.1±0.4	25
Carbaryl	1000	31	5.9	10#	1.3±0.6	13

*Mean weight gain per group during interval between start of experiment and time mice were sacrificed

†Mean ± SD calculated from tumor bearing mice

‡Percentage of mice with tumors times the mean number of tumors per tumor bearing mouse

#NS: no significant difference between control group

TABLE 14. INTERACTION BETWEEN THE CARBAMATE INSECTICIDE CARBARYL IN THE DIET AND THE CARCINOGEN BP (P.O.) ON GROWTH RATE AND NEOPLASIA OF THE LUNG OF A/J MICE

Mice were fed diets containing either 5% corn oil (control) and 3 mg BP given p.o. on the 7th and 21st day of the experiment or 5% corn oil and 1000 ppm carbaryl and 3 mg BP given p.o. on the 7th and 21st day of the experiment. Mice were 9 weeks old at start of experiment and after 16 or 20 weeks on the diet were sacrificed for gross tumor count.

Experimental Group		Duration of Experiment (wk)	Number of Mice	Weight Gain* (g)	Mice with Tumors (%)	Number of Tumors/ Mouse†	Tumorigenic Index‡
Amount of Insecticide Added to Diet (ppm)	Amount of Carcinogen Given Orally						
Experiment 1							
Control I	BP 3 mg p.o. (2X)	20	17	5.6	88	3.7±2.3	326
Carbaryl 1000	BP 3 mg p.o. (2X)	20	18	5.7	100#	5.7±5.9	570
Experiment 2							
Control II	BP 3 mg p.o. (2X)	16	31	6.0	55	1.3±0.8	71
Carbaryl 1000	BP 3 mg p.o. (2X)	16	34	5.7	47	1.4±0.6	66

*Mean weight gain per group during interval between start of experiment and time mice were sacrificed
†Mean ± SD calculated from tumor bearing mice
‡Percent of mice with tumors times the mean number of tumors per tumor bearing mouse
#Significantly different from control I group ($P < 0.05$)

TABLE 15. INTERACTION BETWEEN THE CARBAMATE INSECTICIDE CARBARYL IN THE DIET AND THE CARCINOGEN BP ON BP HYDROXYLASE ACTIVITY OF THE LIVER, LUNG AND FORESTOMACH OF A/J MICE

Mice were fed diets containing one of the following: 5% corn oil (control I), 5% corn oil and 1000 ppm carbaryl, 5% corn oil (control II) and 3 mg BP benzo(a)pyrene given p.o. on the 7th and 21st day of the experiment, or 5% corn oil and 1000 ppm carbaryl and 3 mg BP given p.o. on the 7th and 21st day of the experiment. Mice were 9 weeks old at start of experiment and after 20 weeks on the diet were sacrificed for enzyme assay. The results are expressed as pmoles of 3-hydroxybenzo(a)pyrene per mg of protein per min. Each value represents the mean ± S.D. of 4 determinations.

Experimental Group		BP Hydroxylase Activity		
Amount of Insecticide Added to Diet (ppm)	Amount of Carcinogen Given Orally	Liver	Lung	Forestomach*
Control I	None	39.76±7.45	3.87±0.57	0.099±0.000
Carbaryl 1000	None	45.68±4.27	3.60±0.36	0.084±0.008†
Control II	BP 3 mg p.o. (2x)	42.11±6.14	3.06±0.28	0.102±0.015
Carbaryl 1000	BP 3 mg p.o. (2x)	41.45±6.32	3.86±0.21‡	0.070±0.008#

*Three forestomachs were pooled per determination

†Significantly different from control I group ($P < 0.05$)

‡Significantly different from control II group ($P < 0.005$)

#Significantly different from control II group ($P < 0.02$)

SECTION 4

REFERENCES

1. Ducan, R.M. A Gas Chromatographic Determination of Benzo(a) pyrene Using Electron Capture. Amer. Ind. Hyg. Ass. J. 30: 624-629, 1969.

2. Hoffman, D. and Wynder, E. Smoke of Cigarettes and Little Cigars: An Analytical Comparison. Science 178: 1197-1199, 1972.

3. Kuratsune, M. Benzo(a)pyrene Content of Certain Pyrogenic Materials. J. Natl. Cancer Inst. 16: 1485-1496, 1969.

4. Davis, K.J. Tumorigenic Potential of Aldrin and Dieldrin in Mice. Toxicol. Appl. Pharmacol. 4: 187-189, 1962.

5. Tomatis, L., Turusov, V., Day, N. and Charles, R.T. The Effect of Long Term Exposure to DDT in CF-1 Mice. Int. J. Cancer 10: 489-506, 1972.

6. Dorough, H.W. and Atallah, Y.H. Cigarette Smoke as a Source of Pesticide Exposure. Bull. Environ. Contam. Toxicol. 13: 101-107, 1975.

7. Heidelberger, C. Current Trends in Chemical Carcinogenesis. Fed. Proc. 32: 2154-2161, 1973.

8. Miller, J.A. Carcinogenesis by Chemicals: An Overview - GHA Glowes Memorial Lecture. Cancer Res. 30: 559-576, 1970.

9. Gelboin, H.V. A Microsomal Dependent Binding of BP to DNA. Cancer Res. 29: 1272-1276, 1969.

10. Sims, P., Grover, P.L., Swaisland, H., Pal, K. and Hewer, A. Metabolic Activation of Benzo(a)pyrene Proceeds by a Diol-epoxide. Nature 252: 326-328, 1974.

11. Huberman, E., Sachs, L., Yang, S.K. and Gelboin, H.V. Identification of Mutagenic Metabolites of Benzo(a)pyrene in Mammalian Cells. Proc. Natl. Acad. Sci. (USA), 73: 607-611, 1976.

12. Weinstein, I.B., Jeffrey, A.M., Jennette, K.W., Blobstein, S.H., Harvey, R.G., Harris, C., Kasai, H. and Nakanishi, K. Benzo(a)pyrene Diol-Epoxides as Intermediates in Nucleic Acid Binding in In Vitro and In Vivo . Science 193: 592-594, 1976.

13. Wislocki, P.G., Wood, A.V., Chang, R.L., Levin, W., Yagi, H., Hernandez, O., Jerina, D.M. and Conney, A.H. High Mutagenicity and Toxicity of a Diol-Epoxide Derived from Benzo(a)pyrene. Biochem. Biophys. Res. Commun. 68: 1006-1012, 1976.

14. Wood, A.W., Wislocki, P.G., Chang, R.L., Levine, W., Lu, A.Y.H., Yagi, H., Hernandez, O., Jerina, D.M. and Conney, A.H. Mutagenicity and Cytotoxicity of Benzo(a)pyrene Benzo-Ring Epoxides. Cancer Res. 36: 3358-3366, 1976.

15. Kapitulnik, J., Levin, W., Conney, A.H., Yagi, H., Jerina, D.M. Benzo(a)pyrene 7,8-dihydrodiol Is More Carcinogenic than Benzo(a)pyrene in Newborn Mice. Nature 266: 378-380, 1977.

16. Stevens, J.T., Stitzel, R.E. and McPhillips, J.J. Effects of Subacute Administration of Anticholinesterase Insecticides on Hepatic Microsomal Metabolism. Life Sci. 11: 423-431, 1972.

17. Weber, R.P., Coon, J.M. and Triolo, A.J. Effect of the Organophosphate Insecticide Parathion and its Active Metabolite Paraoxon on the Metabolism of Benzo(a)pyrene in The Rat. Cancer Res. 34: 947-952, 1974.

18. Kinoshita, F.K., Frawley, J.P. and Dubois, K.P. Quantitative Measurement of Induction of Hepatic Microsomal Enzymes by Various Dietary Levels of DDT and Toxaphene in Rats. Toxicol. Appl. Pharmacol. 9: 505-513, 1966.

19. Cress, C.R. and Strother, A. Effects on Drug Metabolism of Carbaryl and 1-Naphthol in the Mouse. Life Sci. 14: 861-872, 1974.

20. Report of the Subcommittee on Environmental Carcinogenesis: General Criteria for Assessing the Evidence for Carcinogenicity of Chemical Substances. J. Natl. Cancer Inst. 58: 461-465, 1977.

21. Nebert, D.W. and Gelboin, H.V. Substrate-Inducible Microsomal Aryl Hydrocarbon Hydroxylase in Mammalian Cell Culture. I. Assay and Properties of Induced Enzyme. J. Biolog. Chem. 243: 6242-6249, 1968.

22. Lowry, O.H., Rosebrough, N.J. and Farr, A.L. Protein Measurement with Folin Phenol Reagent. J. Biolog. Chem. 193: 265-275, 1951.

23. Wattenberg, L.W. Inhibition of Carcinogenic and Toxic Effects of Polycyclic Hydrocarbons by Phenolic Antioxidants and Ethoxyquin. J. Natl. Cancer Inst. 48: 1425-1430, 1972.

24. Shimkin, M.B. Induced Pulmonary Tumors in Mice. II. Reaction of Lungs of Strain A Mice to Carcinogenic Hydrocarbons. Arch. Path. 29: 239-255, 1940.

25. Bross, I.D.J. Is There an Increased Risk? Fed. Proc. 13: 815-819, 1954.

26. Collins, V.J., Gardner, W.V. and Strong, L.C. Experimental Gastric Tumors in Mice. Cancer Res. 3: 29-35, 1943.

27. Kouri, R.E., Ratrie, H. and Whitmire, C.E. Evidence of a Genetic Relationship between Susceptibility to 3-Methylcholanthrene-Induced Subcutaneous Tumors and Inducibility of Aryl Hydrocarbon Hydroxylase. J. Natl. Cancer Inst. 51: 197-200, 1971.

28. Kouri, R.E., Rude, T., Thomas, P.E. and Whitmire, C.E. Studies on Pulmonary Aryl Hydrocarbon Hydroxylase Activity in Inbred Strains of Mice. Chemical Biol. Interactions 13: 317-331, 1976.

29. Kouri, R.E., Salerno, R.A. and Whitmire, C.E. Relationship between Aryl Hydrocarbon Hydroxylase Inducibility and Sensitivity to Chemically Induced Subcutaneous Sarcomas in Various Strains of Mice. J. Natl. Cancer Inst. 50: 363-368, 1973.

30. Nettesheim, B. and Hammons, A.S. Induction of Squamous Cell Carcinoma in the Respiratory Tract of Mice. J. Natl. Cancer Inst. 47: 697-701, 1971.

31. Wattenberg, L.W., Leong, J.L. and Strand, P.J. Benzpyrene Hydroxylase Activity in the Gastrointestinal Tract. Cancer Res. 22: 1120-1125, 1962.

32. Kellerman, G., Shaw, C.R. and Kellerman, M.L. Aryl Hydrocarbon Hydroxylase Inducibility and Bronchogenic Carcinoma. New Engl. J. Med. 289: 934-937, 1973.

33. Tannenbaum, A. and Silverstone, H. The Genesis and Growth of Tumors IV. Effects of Varying the Proportion of Protein (Casein) in the Diet. Cancer Res. 9: 162-173, 1949.

34. Tannenbaum, A. and Silverstone, H. The Influence of the Degree of Caloric Restriction on the Formation of Skin Tumors and Hepatomas in Mice. Cancer Res. 9: 724-727, 1949.

35. Wiebel, F.J., Leutz, J.C. and Gelboin, H.V. Aryl Hydrocarbon (Benzo(a)pyrene) Hydroxylase: Inducible in Extrahepatic Tissues of Mouse Strains not Inducible in Liver. Arch. Biochem. Biophys. 154: 292-294, 1973.

36. Thomas, P.E., Kouri, R.E. and Hutton, J.J. The Genetics of Aryl Hydrocarbon Hydroxylase Induction in Mice: A Single Gene Difference between C57BL/6J and DBA/2J. Biochem. Genet. 6: 157-168, 1972.

37. Johnson, E.L.: Pesticide Products Containing Toxaphene (1976 Experimental Design Status Report: Carcinogenesis Bioassay) Federal Register 42: No. 101, 26859-26920, 1977.

38. MacDonald, J.C., Miller, E.C., Miller, J.A. and Rusch, H.P. The Synergistic Action of Mixtures of Certain Hepatic Carcinogens. Cancer Res. 12: 50-54, 1952.

39. Berenblum, I. A Re-evaluation of the Concept of Cocarcinogenesis. Progr. Exptl. Tumor Res. 11: 21-30, 1969.

40. Gelboin, H.V., Wiebel, F. and Diamond, L. Dimethylbenzanthracene Tumorigenesis and Aryl Hydrocarbon Hydroxylase in Mouse Skin: Inhibition by 7,8-Benzoflavone. Science 170: 169, 1970.

41. Weibel, F.J., Leutz, J.C., Diamond, L. and Gelboin, H.V. Aryl Hydrocarbon (Benzo(a)pyrene) Hydroxylase in Microsomes from Rat Tissue: Differential Inhibition and Stimulation by Benzoflavones and Organic Solvents. Arch. Biochem. Biophys. 144: 78-86, 1971.